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ANTIMICROBIAL ACTIVITIES OF GARLIC AND GINGER EXTRACTS ON SOME CLINICAL ISOLATES

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ABSTRACT

The antibacterial activity of n-hexane and methanol extracts of ginger and garlic was determined *in vitro* against *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae* and *Shigella dysenteriae* using agar well diffusion technique. The phytochemical screening revealed the presence of flavonoids, saponins, carbohydrates, alkaloids and triterpenes in the plant extracts. The n-hexane and methanol extracts of garlic were observed to be more potent against *S. dysenteriae* with maximum zone of inhibition of 27 mm at 40 mg/ml and 29 mm at 80 mg/ml. The n-hexane and methanol extracts of ginger were observed to be more potent against *E. coli* with maximum zone of inhibition of 16 mm at 40 mg/ml and 19 mm at 80 mg/ml. The MIC of the methanolic extract against the test organisms was determined to show values between 5 and 20 mg/ml. In comparison, the n-hexane extract had MIC values between 2.5 and 10 mg/ml. The methanol extract of ginger had MIC values between 10 and 40 mg/ml while the n-hexane had MIC values between 10 and 20 mg/ml. The MBC of methanol garlic extract was between 10 and 40 mg/ml. The n-hexane garlic extract had MBC values between 2.5 and 20 mg/ml. The methanol extract of ginger had MBC values between 10 and 40 mg/ml while the n-Hexane ginger extract had MBC values between 10 and 20 mg/ml. The result of this study showed that the extracts had activity against the test organisms and as such could be used for drug development.

Contribution/Originality: This study is one of the very few studies which have investigated the use of garlic and ginger for their antimicrobial properties aside from their use as spices. These plant-based extracts provide hope for solving antimicrobial resistance problems since it is difficult for microorganisms to develop resistance to them.

1. INTRODUCTION

Ginger, *Zingiber officinale* (commonly called 'jinja' in Igbo, 'cithar' in Hausa and 'Atale' in Yoruba) is an erect, slim herbaceous perennial plant that possess a fleshy and thick underground rhizome and having one or more aerial leafy stems, that grows up to 1.25 m tall. Ginger is grown in the tropical weathers of Australia, West Africa, India, Jamaica Brazil, China, and some parts of the United States (Suruchi *et al.*, 2016). In the first year of growth, it produces a green, straight stalk like stem about 60 cm high growing from the rhizome. Its leaves grow and measures about 12-30 cm long which dies off each year. The crop grows preferably in warm, sunny conditions, and may profit from shade during hot days, especially when young. Shading is however generally considered redundant.

The optimum rainfall is 2500-3000 mm, well-distributed over the year. The odor and taste of ginger plant are aromatic, characteristic and pungent (Shubha, 2015).

Fresh ginger, powdered ginger and preserved ginger are widely used as a spice. Fresh ginger has wide application in cooking, ginger ale and other concoctions. Ground ginger is used mostly for cooking purposes and may also be used as flavor in processed foods. Preserved ginger on the other hand is used in the production of processed foods such as jams, marmalades, cakes and confectioneries (Sharifi-Rad *et al*., 2017). Ginger plants have been used as spice and medicine in China and India for ages. Ginger plants were grown in pots and carried abroad on long sea voyages to prevent scurvy (Suruchi *et al*., 2016). Oil extracted from this plant exhibit antimicrobial activity due to the presence of components such as eugenol, thymol, 1,8-cineole, pinenes, linalool and terpineol (Suruchi *et al*., 2016). Fresh ginger has applications in the treatment of cold-induced diseases, nausea, asthma, cough, colic, heart palpitation, swelling, dyspepsia, loss of appetite and rheumatism. It serves as remedy for asthma and cough when fresh ginger juice is mixed with small quantity of fresh lemon juice and honey (Ponmurugan and Rajaram, 2012). Ginger play important medicinal roles due to the presence of certain constituents such as gingerol, paradol, shogoal, zingerone, zerumbone, terpenoids and ginger flavonoids (Arshad *et al*., 2014).

Allium sativum (commonly called 'aayu' in Yoruba, 'ayo-ishi' in Igbo and 'tafarunua' in Hausa) is a perennial bulbous plant that initially came from middle Asia, and is at present grown globally. Garlic can grow up to 2 feet in height or more. The bulb is the main part of the plant which is used for medicine (Steven, 2015). Each garlic bulb is made up of 4 to 20 cloves. Each garlic clove may weigh about 1 gram in weight. Fresh, aged, dried or garlic can be used garlic supplements. Each of the supplements may have different effects to the body (Sethi *et al*., 2014). It is commonly used as seasoning. It helps prevent certain heart diseases including atherosclerosis, high cholesterol, high blood pressure and boost the immune system as well as protect against cancer (Steven, 2015). The medicinal potency of garlic is due to glycoside, vitamin B, C, and D allisatin II and I. It also contains volatile sulphur oil, which has a vermifugal action (Arshad *et al*., 2014).

Antimicrobial activities of garlic and ginger could be assayed using *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae* and *Shigella dysenteriae*. These microorganisms react to the slightest change in their environment, they tend to multiply rapidly in favorable environments but decrease if the environment becomes unfavorable. The problem of resistance of microorganisms to antimicrobials is a reoccurring problem worldwide. However, plant-based antimicrobials are devoid of many side effects associated with various synthetic antimicrobial compounds (Ehigbai *et al*., 2016).

This research work was therefore aimed at evaluating the antimicrobial activities of garlic and ginger on *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae* and *Shigella dysenteriae*.

2. MATERIALS AND METHOD

2.1. Collection and Identification of the Plant Materials

The plants materials were obtained from Bosso market, Minna, Niger state, Nigeria. They were identified by an Ethnobotanist in the department of Plant Biology, Federal University of Technology, Minna (FUTMinna) as *Zingiber officinale* and *Allium sativum* respectively.

2.2. Preparation of Extracts

The ginger plantwas washed with distilled water to get rid of sand particles and air dried at ambient temperature for six weeks. The garlic bulbs were separated into cloves. The cloves skins were peeled off and the cloves were sliced and also air dried at ambient temperature for about seven weeks. The dried materials were pounded using a sterile laboratory mortal and grinded using a sterile electric blender to obtain a homogenous sample. 120 g of the powdered samples each were then extracted with 750 ml of methanol and n-Hexane by cold maceration method as described by Handa *et al*. (2008). After extracting the plant materials, it was then

concentrated using rotatory evaporator at 40°C. The extract obtained was then freeze dried to remove portion of water present in the extract. It was then stored in sterile sample bottles and preserved in the refrigerator at 4°C until further use. Portions of the plant extracts were then subjected to phytochemical screening.

2.3. Phytochemical Screening

The phytochemical screening for secondary metabolites of the plant extracts were evaluated according to the method of Harborne (1973). The phytochemical screening was done to confirm the presence or absence of certain phytochemical components that are responsible for the antimicrobial activities of plant extracts such as triterpenes, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins and alkaloids.

2.4. Collection of Test Organisms

Clinical isolates of *Escherichia* sp., *Salmonella* sp., *Klebsiella*sp. and *Shigella*sp. were obtained in broth medium from patients attending Old General Hospital, Minna, Niger State, Nigeria and transported in ice packs to the laboratory. The isolates were confirmed subculturing them on MacConkey agar (MCA), Eosine Methylene Blue (EMB) and *Salmonella-Shigella* agar (SSA). The colonies formed were then Gram stained and subsequently subjected to other biochemical tests (Catalase Test, Methyl Red-Voges Proskauer Test, Indole Test, Simmon's Citrate Test, Oxidase Test, Urease Test, Triple Sugar Iron Agar (TSIA)) (Clarke and Cowan, 1952; Daniel, 2000). The pure isolates were then stored on Nutrient agar slant bottles at a temperature of 4°C until further use.

2.5. Standardization of Organism

The method of James *et al.* (1983) was employed in the standardization of the test organisms. 20 ml of sterile nutrient broth was inoculated with the test organism for 24 h at 37°C. 0.2 ml of the 24 h culture was subcultured into another 20 ml of sterile nutrient broth and incubated at 37°C for 5 h to standardize the culture to 10^6 cfu/ml. The standardized culture was then used subsequently to evaluate the antibacterial of the plant extracts against the test organisms.

2.6. Antibacterial Assay

The agar well diffusion method was employed to determine the antibacterial activity of the plant extracts according to the Clinical and Laboratory Standards Institute (CLSI) (2006). The standardized suspension was used to inoculate the surfaces of sterile nutrient agar plates using sterile cotton swab. 8 mm diameter wells were bored using sterile core borers in the solidified agar. The bottom of the wells was then closed using 1 ml of sterile nutrient agar. The wells were then filled with desired concentrations of the plant extracts (40 mg/ml and 80 mg/ml). The plates were allowed to stand for about 3 h at room temperature for the extracts to diffuse and then the agar plates were incubated at 37°C for 24 hours. The antibacterial activities of the plant extracts were evaluated by appearance of zones of inhibition around the wells while lack of activity was observed by absence of zones of inhibition. The antibacterial activity of the plant extracts was compared with amoxicillin (0.25µg/ml) to check its effectiveness. The control plates included extract sterility control (ESC), medium sterility control (MSC) and organism viability control (OVC).

2.7. Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined according to the CLSI (2006). The plant extract was dissolved in 5 ml of solution (0.5 ml DMSO and 4.5 ml of water). 2 ml sterile nutrient broth was transferred into 5 different test tubes and 2 ml of different concentration of extract was added respectively. The test organism was inoculated into the labeled tubes with the exception of the negative control. The tubes were incubated at 37°C for 24h. This procedure was repeated

for the remaining extracts and test organisms. The MIC was taken as the lowest concentration that prevented any visible growth.

2.8. Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined according to the CLSI (2006). The test tube that showed no visible growth was subcultured onto sterile nutrient agar and incubated at 37°C for 24 h. The least concentration at which the organism did not grow was taken as the minimum bactericidal concentration.

3. RESULTS

3.1. Phytochemical components of Zingiberofficinaleand Allium sativumplants

The garlic extract obtained was a golden-yellow, gummy residue with a pungent offensive smell while that of ginger was brown with spicy-sweet smell. Result of the preliminary phytochemical screening of n-Hexane and methanolic extracts of ginger and garlic is presented in Table 1. Flavonoids, saponins and triterpenes were present in the ginger extracts. However, the methanol extract of ginger contained carbohydrates which was absent in the n-Hexane extract of ginger. Phenol, tannins, alkaloids, cardiac glycosides and steroids were absent in both n-Hexane and methanolic extract of ginger. Flavonoids, saponins, alkaloids and triterpenes were present in n-Hexane and methanolic extracts of garlic. Carbohydrates were found present in methanolic extract of garlic but absent in the n-Hexane extract. However, phenol, tannins, steroids and cardiac glycosides were absent. In general, phenol, tannins, steroids and cardiac glycosides were absent in all four extracts.

Table-1. Preliminary qualitative phytochemical analysis of n-Hexane and Methanol extracts of garlic and ginger extracts.

Phytochemical	Hex_{garlic}	Met_{garlic}	Hex_{ginger}	Met_{ginger}
Phenol	-	-	-	-
Flavonoids	+	+	++	+++
Tannins	-	-	-	-
Saponins	++	++	++	++
Alkaloids	+	+	-	-
Carbohydrates	-	+++	-	++
Steroids	-	-	-	-
Triterpenes	++	++	+++	++
Cardiac glycosides	-	-	-	-

KEY: +++: highly present, ++: moderately present, +: Low, -: absent, Met_{garlic} = Methanolic extract of garlic, Hex_{garlic} = n-Hexane extract of garlic, Met_{ginger}= Methanolic extract of ginger,Hex_{ginger} = n-Hexane extract of ginger.

3.2. Antibacterial Activity

The antibacterial activity of the crude plant extracts against the clinical isolates tested is summarized on Table 2. Methanol (Met_{garlic}), n-Hexane (Hex_{garlic}), methanol (Met_{ginger}) and n-Hexane (Hex_{ginger}) plant extracts were active against the test organisms at 40mg/ml. However, the n-Hexane and methanolic garlic extracts showed more activity. Amoxicillin, the standard drug for treating the enteric pathogens inhibited the growth of the test organisms at a standard concentration of 0.25 µg/ml.

Table-2. Antibacterial activity of n-Hexane and methanolic extracts of ginger and garlic against clinical isolates.

Clinical Isolate	Concentration of extracts (Mg/ml) / Zone of inhibition (mm)								Control
	Met_{garlic}		Hex_{garlic}		Met_{ginger}		Hex_{ginger}		
	40	80	40	80	40	80	40	80	0.25 µg/ml
Shigella sp.	27.0	29.0	21.0	24.0	7.0	8.0	12.0	17.0	31.0
Escherichia sp.	25.0	28.0	26.0	30.0	10.0	15.0	16.0	19.0	29.0
Salmonella sp.	22.0	25.0	19.0	22.0	11.0	13.0	9.0	12.0	30.0
*Klebsiella*sp.	23.0	25.0	21.0	23.0	9.0	13.0	14.0	15.0	31.0

KEY: Met_{garlic} = Methanolic extract of garlic, Hex_{garlic} = n-Hexane extract of garlic, Met_{ginger}= Methanolic extract of ginger,Hex_{ginger} = n-Hexane extract of ginger.

3.3. Minimum Inhibitory Concentration (MIC) of Methanolic and N-Hexane Garlic and Ginger Extracts

The methanol extract had MIC values of 10 mg/ml, 5 mg/ml, 20 mg/ml and 10 mg/ml against *Shigella*sp., *Escherichia* sp., *Salmonella* sp., and *Klebsiella*sp.respectively. The n-Hexane extract had MIC values of 5 mg/ml, 5 mg/ml, 10 mg/ml and 2.5 mg/ml against*Shigella*sp., *Escherichia* sp., *Salmonella* sp., and *Klebsiella*sp.respectively. In contrast, the methanol extract of ginger had MIC values of 20 mg/ml, 10 mg/ml, 40 mg/ml and 10 mg/ml against*Shigella*sp., *Escherichia* sp., *Salmonella* sp., and *Klebsiella*sp.respectively. The n-Hexane had MIC values of 10 mg/ml, 10 mg/ml, 20 mg/ml and 20 mg/ml against*Shigella*sp., *Escherichia* sp., *Salmonella* sp., and *Klebsiella*sp.respectively. Garlic extracts however, had the lowest MIC value (2.5 mg/ml) suggesting that it has more activity compared to the ginger extracts. The MIC of the plant extracts is summarized in Table 3.

Table-3. Minimum Inhibitory Concentration (MIC) of Methanolic and n-Hexane garlic and ginger extracts.

Clinical isolate	MIC (mg/ml)			
	Met_{garlic}	Hex_{garlic}	Met_{ginger}	Hex_{ginger}
*Shigella*sp.	10	5.0	20	10
Escherichia sp.	5.0	5.0	10	10
Salmonella sp.	20	10	40	20
*Klebsiella*sp.	10	2.5	10	20

KEY: Met_{garlic} = Methanolic extract of garlic, Hex_{garlic} = n-Hexane extract of garlic, Met_{ginger}= Methanolic extract of ginger,Hex_{ginger} = n-Hexane extract of ginger.

3.4. Minimum Bactericidal Concentration (MBC) of Methanolic and N-Hexane Garlic and Ginger Extracts

The methanol garlic extract had MBC values of 10 mg/ml, 10 mg/ml, 40 mg/ml and 10 mg/ml against *Shigella*sp., *Escherichia* sp., *Salmonella* sp., and *Klebsiella*sp.Respectively Table 4. The n-Hexane garlic extract had MBC values of 5 mg/ml, 10 mg/ml, 20 mg/ml and 2.5 mg/ml against *Shigella*sp., *Escherichia* sp., *Salmonella* sp., and *Klebsiella*sp.respectively. In contrast to the ginger extracts however, the methanol extract of ginger had MBC values of 40 mg/ml, 20 mg/ml, 40 mg/ml and 10 mg/ml against the isolates respectively. The n-Hexane ginger extract had MBC values of 20 mg/ml, 10 mg/ml, 20 mg/ml and 20 mg/ml against the isolates respectively. The MBC values of garlic extracts when compared to that of ginger extracts are considerably low suggesting that garlic had higher activity *in vitro* against the test organisms. Some of the concentrations had the same MIC and MBC values.

Table-4. Minimum Bactericidal Concentration (MBC) of Methanolic and n-Hexane garlic and ginger extracts.

Clinical isolate	MBC (mg/ml)			
	Met_{garlic}	Hex_{garlic}	Met_{ginger}	Hex_{ginger}
*Shigella*sp.	10	5.0	40	20
Escherichia sp.	10	10	20	10
Salmonella sp.	40	20	40	20
*Klebsiella*sp.	10	2.5	10	20

KEY: Met_{garlic} = Methanolic extract of garlic, Hex_{garlic} = n-Hexane extract of garlic, Met_{ginger}= Methanolic extract of ginger,Hex_{ginger} = n-Hexane extract of ginger.

4. DISCUSSION

The organisms tested were all susceptible of varying concentrations of the plant extracts. The antibacterial activity of methanol and n-hexane extracts of ginger and garlic is attributed to the presence of bioactive components (Ponmurugan and Rajaram, 2012; Dixon and Jeena, 2017). The presence of flavonoids, carbohydrates, saponins, triterpenes and alkaloids present in the extracts were consistent with those of previous studies (Cheeke, 1989; Abdullahi *et al.*, 2014; Aliyu *et al.*, 2017). However, phenol, tannins, steroids and cardiac glycosides were absent in all four extracts.

The garlic extracts gave the widest zones of inhibition compared to the ginger extracts against all the bacterial isolates. The activity of these plants may be attributed to the presence of secondary metabolites within them (Patra and Saxena, 2009). It was also observed that the solvent of extraction affected the degree of sensitivity of the test organisms as reported by Abdullahi *et al.* (2014). The antibacterial activity of ginger extracts was generally low, however, Aliyu *et al.* (2017) reported that ginger extract demonstrated greater antibacterial activity against a variety of bacteria, although mixed result is attributed to different ginger preparations and varying strengths. The low activity shown by ginger extracts may also be due to the fact that the active components in the extracts may not have had activity *in vitro* against the clinical isolates or that the concentrations used may not have been high enough to cause antimicrobial effects against the organisms as reported by Cheeke (1989) and Aliyu *et al.* (2017).

High minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) invariably means low activity, whereas low MIC and MBC values means high activity of the plant material. In this study however, garlic extracts had low MIC values as compared to those of ginger extracts when test against the bacterial isolates. However, n-hexane garlic extract had the lowest MIC value (2.5 mg/ml) against *Klebsiella* sp. whereas, the methanolic garlic extract had the highest MIC value of 20 mg/ml against *Salmonella* sp. In contrast, the methanolic ginger extract also had the highest MIC value of 40 mg/ml against *Salmonella* sp. The lowest MIC value for the extract was however, 10 mg/ml. This result is in accordance with the findings of Iram *et al.* (2012) and Ponmurugan and Rajaram (2012).

Both extracts had the same MBC values on two of the isolates. The methanolic and n-hexane garlic extracts had MBC values of 40 and 20 mg/ml respectively against *Salmonella* sp., whereas the methanolic and n-hexane ginger extracts had the same values against *Shigella* sp. The garlic extracts were cidal against *Shigella* sp., and *Klebsiella* sp. The ginger extracts were also cidal against *Salmonella* sp., and *Klebsiella* sp. but only the n-hexane ginger extract was cidal against *Escherichia coli*. MBC values of garlic extracts when compared to that of ginger extracts are considerably low suggesting that garlic had higher activity *in vitro* against the test organisms as reported by Aliyu *et al.* (2017). The n-hexane extracts were found to be more potent than the methanolic extracts which is in contrast to findings by Garba *et al.* (2013). This accounts for the effect of the solvent system, which greatly affects the antibacterial activity of the crude extracts.

5. CONCLUSION

This study showed that *S. dysenteriae*, *S. enterica*, *E. coli* and *K. pneumoniae* were susceptible to the ginger and garlic extracts which invariably means that the plants have antibacterial activity and could be a source of active antimicrobial agent for the development of drugs for the treatment of these infectious microorganisms.

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